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SCREENING OF ANTIMICROBIAL POTENTIAL AND BIOACTIVE COMPONENTS OF SELECTED MEDICINAL PLANTS AGAINST INFECTIOUS BACTERIAL ISOLATES FROM LEUKEMIA PATIENTS

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Blood cancer

Nigella sativa

Phenolic compounds

ABSTRACT

In the present study, an attempt was made to understand the occurrence of secondary infections in immuno-suppressed blood cancer patients and herbal suppression of these infections. The antibacterial potential of the ethanolic and methanolic crude extracts of ten medicinal plants against five clinical microbial isolates of blood cancer cases were evaluated through disc diffusion assay. Among the tested plants *Nigella sativa* extract showed highest antimicrobial potential followed by *Lawsonia inermis*, while *Ficus carica* exhibited least antimicrobial activity. The maximum zone of inhibition (16mm) was observed against *Streptococcus viridans* by the extract of *Nigella sativa*. Phenolics analysis by high performance liquid chromatography ultraviolet detector (HPLC-UV) revealed that parahydroxy benzoic acid was the major phenolic acid found in *Olea europaea* (797.8ng/g) and *Mentha longifolia* (367.98ng/g) while highest concentration of gallic acid was reported from *L. inermis* (3936.86ng/g) and vanillic acid (1942.51ng/g) in *Ficus carica*. These results indicated that phenolic compounds play an important role in pathogenic bacteria's cell growth inhibition and could be used as future antimicrobial and chemotherapeutic agent. Further, *N. sativa*, *L. inermis*, *Boswellia sacra* and *Trigonella foenum-graecum* plant extracts showed highest antimicrobial potential against microbial isolates isolated from blood cancer patients.

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1 Introduction

Cancer is now the third leading cause of death worldwide, with >12 million new cases and 7.6 million cancer deaths estimated to have occurred (Garcia et al., 2007; Karpinski & Adamczak, 2018). By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year (Boyle & Levin, 2008; Hanahan & Weinberg, 2011). Moreover, the global distribution of cancer and types of cancer that predominate continues to change, especially in economically developing countries. Low and middle income countries accounted about half (51%) of all cancers worldwide in 1975; this proportion increased to 55% in 2007 and projected to reach 61% by 2050 (Worldwide Cancer Statistics, 2018). According to Bray & Moller (2006) cancer particularly leukemia patients are highly susceptibility against bacterial and fungal infections. These infections might be an important cause of mortality and morbidity for febrile neutropenic cancer patients. In recent years, the introduction of novel chemotherapeutic bio factors and use of bio agents chemotherapeutic ways for leukaemia patients have improved the stand point of this disease, because a large number of patients are alleviating their clinical health by this biotherapy. But such intense chemotherapy with cytotoxic drugs usually results in myelosuppression and as a resultant high risk of neutropenia (absolute neutrophil count $< 0.5 \times 10^9/L$) (Bow et al., 2006). It has a damaging effect on the normal human skin and mucosa, which leads to the risk of persistent bacterial, viral and fungal infections as phagocytic activity of the neutrophils impairs by neutropenia (Hotchkiss & Karl, 2003). The patients diagnosed with malignancies and getting chemotherapy, found at higher risk of neutropenia and resultant all kind of infections and thus treated as immunocompromised cases (Groll & Walsh, 2002).

The development of novel techniques in curative arena, particularly after the threat of drug resistance emergence, the use of conventional medicines and the plant-based herbal treatments have been focused and reconsidered as a great source of chemotherapeutic bioaids in all health systems of humans and animals throughout the world (Mahima et al., 2013).

The use of plants in medicine ranges from crude preparations or extracts, to refined extracts and individual bioactive compounds. Approximately 50% of 850 small molecules introduced as drugs over the last 20 years were derived from medicinal plants, plant-derived or plant mimetics, meaning that the synthetic medicinal compounds were discovered from the study of plants (Fowler, 2006). An extensive program at the National Cancer Institute (NCI) screened around 114,000 extracts from an estimated 35000 plant samples for anticancer activity (Balunas & Kinghorn, 2005).

Today, the use of plant drugs has been accepted all over the world. About 57% of the top-selling prescription in the USA contains natural products or derivatives, and one out of three Americans consumes herbal drugs (Newman & Cragg, 2007). In developing countries, the use of medicinal plants has significantly increased due to the low income of the population. About 80% of the people are dependent, wholly or partially, on plant-based drugs (Kuete et al., 2010; Kumari et al., 2018).

Plant derived medicines are usually considered as relatively more safer than synthetic drugs, these have less side effects, intense therapeutic cure and more cost effectiveness (Sher & Hussain, 2009; Iqbal et al., 2017). In wild plants, the lack of infective diseases is the great indication of their successful defense mechanisms against diseases. The defenses mechanisms of these plants are associated to the presence of a huge number of biochemical compounds like alkaloids, phenols, terpenes derivatives compounds and many other antimicrobial agents. Anti microbial compounds that make the essential oils from plants are more précised and evolved in their defensive mode of action against a wide range of pathogenic microorganisms. Thus, the essential oils from plants could have been used as alternative and improved supplements for the treatments of pathogenic infections (Akthar et al., 2014). Keeping in view the potential of medicinal plants as antimicrobial agent against clinical bacterial isolates of cancer patients, the objective of this study was to understand and characterize the bacterial clinical isolates/infections isolated from immuno-suppressed cancer patients through *in vitro* antimicrobial activity, compare the inhibitory effect of ten medicinal plants and to identify phenolics acids of the crude extracts of the plants using High-performance liquid chromatography ultraviolet detector (HPLC-UV).

2 Materials and Methods

The goal of *in vitro* antimicrobial activity testing is to provide a reliable predictor of an organism is likely to respond to sample test in the infected host.

2.1 Selection of medicinal plants for this study

Ten medicinal plants were collected from different regions of Saudi Arabia for this study, *Nigella sativa* (seeds), *Ficus carica* (leaves), *Lepidium sativum* (seeds), *Lawsonia inermis* (leaves), *Azadirachta indica* (leaves), *Boswellia sacra* (resins), *Ocimum basilicum* L. (leaves), *Mentha longifolia* L. (leaves), *Trigonella foenum-graecum* L. (seeds) and *Olea europaea* L. (leaves). The collected plants were identified in the laboratory by comparison of flora which made according to different references concerning with the medicinal plants (Royal Botanic Gardens Victoria, Indiana Native Plant & Wildflower Society).

2.2 Preparation of plant crude extracts

The seeds, leaves and resins plant parts were thoroughly washed to remove debris and the earth remains and allowed to dry under shade. The dried parts were grinded to fine powder. The plant extracts were prepared using the methanol and ethanol solvents. Air-dried plant extracts powder (25g) was taken in 100 ml of 96% methanol and ethanol as a solvent for 24 hrs in a water bath shaker (37°C) and then the extract was filtered with whatman no.1 filter paper and stored in air-tight amber bottles in refrigerator for further use as described by Jameela et al. (2011).

2.3 Preparation of microbial inoculums

Forty-five bacterial isolates were collected from patients with infection from the radiotherapy unit of King Abdulaziz Medical City, Jeddah, Saudi Arabia. All the bacterial isolates were identified using API (Analytical Profile Index) system with biomerieux vitek 2.

Stock bacterial cultures of clinical isolates were maintained at 4°C on nutrient agar slants until the antimicrobial assay. For culturing isolates, nutrient agar slants were prepared using yeast extract (2g), peptone (5 g), NaCl (5g), meat extract (1g), agar (15g) and distilled water (1L). The final pH of prepared medium was adjusted to 7.0. All studied bacterial isolates which have 10⁴ CFU mL⁻¹ used as final inoculum. Five clinical isolates of blood cancer cases (*Salmonella* group B, *Corynebacterium*, *Staphylococcus aureus*, *Klebsiella Pneumoniae* and *Streptococcus Viridans*) were selected on the basis of random occurrence in samples for further studies. Active cultures for selected bacterial isolates were prepared by transferring a sterile loopful swap of culture to 5 ml of nutrient broth and incubated at 37°C for 72 hrs on shaker with 170rpm in order to make a bacterial suspension as described by Jayaram et al. (2004).

2.4 Preparation of crude plants extract

Dried and pounded plants powder (25mg) were dissolved in 99.8% methanol or ethanol (100 ml) using a conical flask and plugged it with sterile cotton ball. The samples were placed for 24 hrs on shaker at 37°C and for another 24 hrs at room temperature as described by Doss & Pugalenti, (2012). The extracts were filtered using whatman no.1 filter paper. The plant extracts were evaporated to dryness using rotary vacuum evaporator at 60°C under reduced pressure. After complete vacuum evaporation, plant extracts were dissolved in 0.25% Dimethyl Sulphoxide (DMSO), and stored at 4°C for further use (Dineshbabu et al., 2015). The solvent DMSO (2.5%) that would not inhibit growth of the microorganisms was used as the negative control for all the experiments (Zgoda & Porter, 2001).

2.5 Paper disc diffusion assay

The initial screening of the extracts for antibacterial activity was conducted by the disc diffusion assay. The dried plant extracts (25mg) were dissolved in dimethyl sulfoxide (DMSO) with a concentration of 2.5 mg/ml. Inoculums of testing bacterial isolates was spread on Muller Hinton Agar (MHA) medium by a sterile inoculation loop, the loop is streaked in at least three directions over the surface of the nutrient agar to obtain uniform growth according to the standard protocol described by the National Committee of Clinical Laboratory Standards (concentration 200 mg/ml). The plates were dried for 5 minutes approximately before use. Sterile paper discs (6 mm in diameter) were impregnated with 30µl of each plant extract and placed on the surface of Mueller Hinton agar plates, that already inoculated with testing bacterial isolates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The plates were subsequently incubated at 37°C for 24 hrs. After incubation each plate was examined and the growth inhibition zones were quantified by measuring the diameter of the zone of inhibition in mm starting from the disc to the end of the clear zone. Filter paper discs containing dimethyl sulfoxide (DMSO) 25 % without any test compounds served as a control and no inhibition was observed. Each assay was analyzed and recorded in triplicate according to method described by Kumara et al. (2009).

2.6 Standard antibiotic analysis

Mueller-Hinton agar plates that have been already inoculated with tested pathogenic bacteria were used to analyze the antimicrobial potential of standard discs of Ciprofloxacin antibiotic (30µg/disc). This antibiotic then further used as standard control in disc diffusion assay with plant extracts. During incubation, the antibiotic diffused away from discs creating a concentration gradient which appeared as inhibition zone around these discs. Inhibition zone diameters (mm) were observed after 18 hrs of incubation at 37°C.

2.7 Analysis of total phenolics

Total phenolics content was estimated by the Folin- Ciocalteu method (Pourmorad et al., 2006). In this, 0.5 ml of plant extracts or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml) and aqueous Na₂CO₃ (4 ml, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenolics values were presented in unit of mg equal gallic acid in 1 g dry plant powder.

2.8 Analysis of total flavonoids

Total flavonoids content was determined by aluminium chloride method (Pourmorad et al., 2006). Plant extracts (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The plant extracts were kept at room temperature for 30 minutes to stabilize the mixture. The absorbance of the reaction mixture was measured with a spectrophotometer at 415 nm. Quercetin was used as a standard estimation for calibration curve. Total flavonoids values were recorded in the unit of mg equal quercetin in 1 g dry plant powder.

2.9 High Performance Liquid Chromatography (HPLC) analysis of phenolic compounds

High performance liquid chromatography analysis with UV detection was performed for the estimation of the phenolic compounds in the plant extracts. The shaded dried plant material (200g) was crushed to make it coarse powder. The coarse powder (20 g) was grinded with 25 ml distilled water of 2 N-HCl. The grinded plant extracts were heated in water bath using air condenser at 100°C for 1 h. The plant extracts were filtered using whatman filter paper No.1. By using a separating funnel, the filtrate was extracted with diethyl ether. The layer of diethyl ether was washed and separated with distilled water and dried over sodium sulphate (anhydrous). The final evaporated extract was obtained using rotary vacuum evaporator at 25°C. The collected extract re-dissolved in HPLC grade ethanol (5ml), prior to the injection into HPLC column. The samples were filtered through 0.22µm organic filter (Millipore) before use (Joshi, 2011).

2.9.1 Analyses of individual phenolic acids by HPLC

The reverse phase high performance liquid chromatography (RP-HPLC) analysis was performed for the estimation of phenolic acids compounds like gallic acid, parahydroxy benzoic acid, vanillic acid, syringic acid and ferulic acid. During study, used HPLC apparatus was HPLC-Beckman model-322 equipped with 100A model pump, 210 injector, 420 controller, mixer and BD-40 recorder. C18 column (ultrasphere) with specification of 5µm (25 cm x 4.6 mm length). Mobile phase was setup strictly with this ratio, methanol: water (1% acetic acid in 20: 80 v/v). Prior to use in HPLC, mobile phase was degased. Flow rate was maintained at 1ml min⁻¹ with chart speed 1cm min⁻¹. UV detector was fixed with max 280 nm λ, aufs Attenuation (0.02) and isocratic mode. For individual phenolic compound, the detector response was calibrated and measured with standard phenolic acids strictly as described by Tandon et al. (2001). All standard phenolic compounds were procured from Sigma-Aldrich chemical company, USA.

2.10 Statistical Analyses

All measurements were performed in triplicate and the results were represented as mean ± SEM. Multiple comparisons of means were performed by men whitney test. In all data analyses a value of P< 0.05 was considered as statistically significant. All computations were made by employing the statistical software (SPSS, version 11.0).

3 Results

3.1 Evaluation of Antimicrobial Potential of selected Medicinal Plants parts

The goal of antimicrobial activity test was to estimate the antimicrobial potential of selected medicinal plants against isolated bacteria. Antimicrobial activity was recorded in the diameter (mm) of inhibitory zone formed around the disc (Table 1). Among methanol crude extracts *N. sativa* showed maximum inhibitor activity (16 mm) against *Streptococcus viridans* (Figure 1) while *L. sativum* showed minimum inhibitor activity (2mm) while in case of ethanol extract *L. inermis* had highest antimicrobial activity (9mm) against *S. viridans* as compared to other plants, while *F. carica* had less activity (0.25mm) against *Staphylococcus aureus*. The control DMSO discs showed no inhibitory zones. Among various tested microorganisms, *Klebsiella pneumoniae* has been shown highest resistant against the used plant extracts and antibiotics.

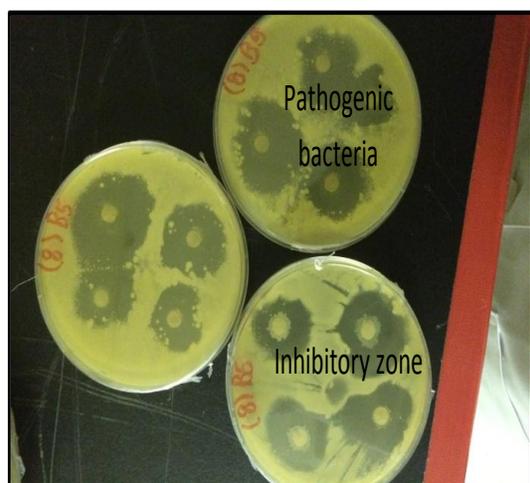
The good antimicrobial potential was also depicted by methanolic extract of *B. sacra*, *T. foenum-graecum* and *O. basilicum* as compared to ethanol extracts. The largest inhibition zones of *B. sacra* were observed for methanol extract against *S. viridian* with 12.4 mm and *Salmonella* Group B 9.75mm zone size respectively. The methanol extract of *T. foenum-graecum* showed inhibition zone (11.8mm) against *Salmonella* Group B while the zone of inhibition against *Corynebacteria* isolates was found (11.5mm).

The highest effect of ethanol extract against *S. viridian* was observed with 6.4 mm zone of inhibition. Methanol extract of *O. basilicum* showed maximum inhibition zone (3mm) against *Corynebacteria*. High antimicrobial activity for ethanol extract of *M. longifolia* and *O. europaea* were recorded as compared to methanol extracts of these plants. The ethanol extract of *M. longifolia* against *Staphylococcus aureus* showed 7mm zone of inhibition and for *Corynebacteria* (4mm) respectively. The methanol extract showed antimicrobial effect against *S. viridian* with 3.6mm zone of inhibition. The inhibition zone against *Corynebacteria* for ethanol extract of *Olea europaea* was observed (4mm) and *Staphylococcus aureus* (9mm). While the methanol extracts depicted only 4mm inhibition zone against *Streptococcus viridian* and 3mm for *Corynebacteria* isolates.

Table 1 The antimicrobial activity of different parts of medicinal plants extracts against cancer clinical bacterial isolates

Bacteria	Inhibition zone (mm)												Antibiotic Ciprofloxacin (30mg/disc)								
	<i>N. sativa</i>		<i>F. carica</i>		<i>Lepidium sativum</i>		<i>L. inermis</i>		<i>A. indica</i>		<i>B. saera</i>			<i>O. basilicum</i>		<i>M. longifolia</i>		<i>T. fragracum</i>		<i>O. europaea</i>	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	
<i>Salmonella</i> group B	1±0.1	1.5±0.4	1.5±0.5	ND	ND	0.5±0.2	7.2±1.5	5±0.1	2.6±0.5	3.8±0.9	9.75±1.5	4.6±1.4	ND	1.76±0.25	2.5±1.0	2.0±1.0	11.8±2.0	2.3±0.5	1.0±0.9	2.0±0.7	9
<i>Corynebacterium</i>	11.7±3.7	9.3±2.2	1.7±1.1	0.5±0.4	2±0.5	ND	8.6±0.5	4.6±0.5	1.5±0.4	4±1.6	9.6±2.6	4.0±1.6	3.0±0.8	1.9±0.75	3.3±1.2	4.0±1.0	11.5±1.2	4.0±1.6	3.0±0.4	4.0±0.9	9
<i>Staphylococcus</i> <i>aureus</i>	15.9±1.3	6.9±0.4	7±2.6	0.25±0	5.4±1.2	5±1.2	8±2.2	5.3±1	5±0.4	ND	6.5±1.3	7.75±0.6	ND	1.3±0.57	ND	7.0±1.6	6.0±0.2	5.8±1.6	1.5±0.5	3.0±0.9	10
<i>Klebsiella</i> <i>pneumonia</i>	ND	ND	2.1±1.1	0.6±0.1	2.7±0.4	ND	7±1.2	4.2±0.2	1.7±0.2	3±0.1	4.0±1.0	4.0±2.0	1.5±0.5	2.4±0.5	1.0±0.2	3.6±0.5	1.0±0	5.3±1.4	1.0±0	1.75±0.4	6
<i>Streptococcus</i> <i>viridians</i>	16±1.2	6.8±1.2	ND	1.3±1	2±0.4	ND	8.9±0.1	9±0.6	3±0	3.4±0.5	12.4±2.6	4.0±0.9	1.0±0.2	ND	3.6±0.5	3.25±1.2	10.0±1.6	6.4±1.2	4±0.5	9.0±1.0	7

Data showed the mean inhibition zone from a triplicate; Mean±SD; E: ethanol extract; M: methanol extract; ND: No inhibition zone; SD: standard deviation; Ciprofloxacin was used as positive control.



a. Ethanol extract against *Corynebacterium*



b. Methanol extract against *Corynebacterium*



c. Ethanol extract against *Streptococcus viridans*



d. Methanol extract against *Streptococcus viridans*



e. Ethanol extract against *Staphylococcus aureus*



f. Methanol extract against *Staphylococcus aureus*

Figure 1 Antimicrobial activity of *Nigella sativa* extracts (zone of inhibition).

3.2 Analyses of total phenolics and total flavonoids

Phenols and flavonoids are the most wide spread secondary metabolites in plant kingdom. These two groups have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator (Rice-Evans et al., 1995). The present study showed that the leaves and seed extracts of all selected plants contained significantly higher amount of phenolics and flavonoids compounds. Total phenolics content ranged from 8.651 ± 0.321 to 5232 ± 0.231 mg GAE/g (gallic acid equivalent) while flavonoids

content ranged between 4.023 ± 0.70 and 2282 ± 0.45 mg quercetin equivalent per g extracts (Table 2).

3.3 Analyses of individual phenolic acids by HPLC

The presence of phenolic compounds were identified by the comparison of their retention times and UV spectra to those of authentic standards analyzed under identical conditions. Qualitative and quantitative analysis of phenolic acids from ten medicinal plants depicted that gallic acid and ferulic acid were detected in all tested plants with different concentrations (Table 3).

Table 2 Total phenolic and flavonoid contents in ten tested medicinal plant extracts (n=3)

Plant crude extracts samples	Plant parts investigated	Total phenolics (mg/g GAE) A	Total flavonoids (mg/g QE) B
<i>Nigella sativa</i>	seeds	116.09±0.30	194.04±11.65
<i>Ficus carica</i>	leaves	52.296 ± 5.23	14.38 ± 0.33
<i>Lepidium sativum</i>	seeds	8.651 ± 0.32	4.023 ± 0.081
<i>Azadirachta indica</i>	leaves	43.7 ± 0.06	529.5 ± 0.14
<i>Lawsonia inermis</i>	leaves	5232.4 ± 0.23	107.33 ± 0.12
<i>Boswellia sacra</i>	resins	315.97 ± 10.04	37.19 ± 11.04
<i>Ocimum basilicum</i>	leaves	43.56±2.13	4.21±0.70
<i>Mentha longifolia</i>	leaves	45.25±0.17	25.17±11.2
<i>Trigonella foenum-graecum</i>	seeds	1813 ± 0.32	2,282±0.45
<i>Olea europaea</i>	leaves	103.99 ± 0.04	79.71 ± 0.08

*All values are expressed as mean ± SEM for three determinations indicates significance at $p < 0.05$;
A mg gallic acid equivalents (GAE)/g extract; B mg quercetin equivalents (QE)/g extract

Table 3 HPLC analyses of individual phenolic acids in selected medicinal plants

Plant crude extracts samples	Gallic acid (ng/g)	Parahydroxy benzoic acid (ng/g)	Vanillic acid (ng/g)	Syringic acid (ng/g)	Ferulic acid (ng/g)
<i>Nigella sativa</i>	12.58	ND	ND	5.66	5.58
<i>Ficus carica</i>	1172.79	1814.18	1942.51	445.54	236.88
<i>Lepidium sativum</i>	896.37	595.03	ND	ND	ND
<i>Azadirachta indica</i>	1548.42	261.04	ND	ND	38.71
<i>Lawsonia inermis</i>	3936.86	26.97	23.36	1201.88	ND
<i>Boswellia sacra</i>	138.82	ND	ND	ND	10.14
<i>Ocimum basilicum</i>	547.02	24.27	ND	ND	236.75
<i>Mentha longifolia</i>	311.72	367.98	174.02	21.76	48.92
<i>Trigonella foenum-graecum</i>	244.87	65.73	ND	21.52	129.57
<i>Olea europaea</i>	397.91	797.8	140.42	ND	30.45

ND=not detected; concentration of samples are in ng/g=ppb;

The chromatogram (Figure 2) showed that one of the most abundant metabolites which have been reported from the methanolic extract of *N. sativa* seeds was gallic acid with retention time $t_R = 3.46$ min and para-hydroxybenzoic acid with retention time $t_R = 10.43$ min.

HPLC analysis of the crude leaves extract of *F. carica* showed the

presence of gallic acid with retention time $t_R = 3.54$ min, protocatechuic acid with retention time $t_R = 6.31$ min, vanillic acid with retention time $t_R = 12.42$ min, syringic acid with retention time $t_R = 13.31$ min, para-cumeric acid with retention time $t_R = 22.03$ min. The separation of the leaves crude extract of *L. inermis* compounds (Figure 3) occurred in the order listed (with the retention times given in parenthesis): gallic acid (3.51min), para-

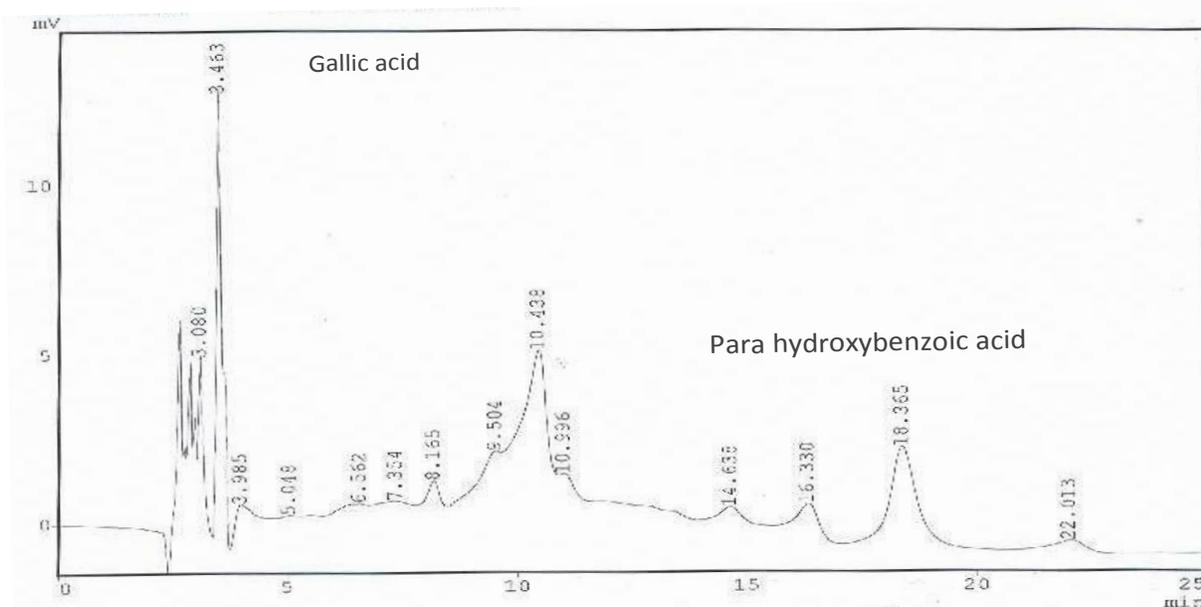


Figure 2 HPLC spectrum of the seeds crude extract of *Nigella sativa*/ black seed at 280 nm absorbance using methanol as solvent.

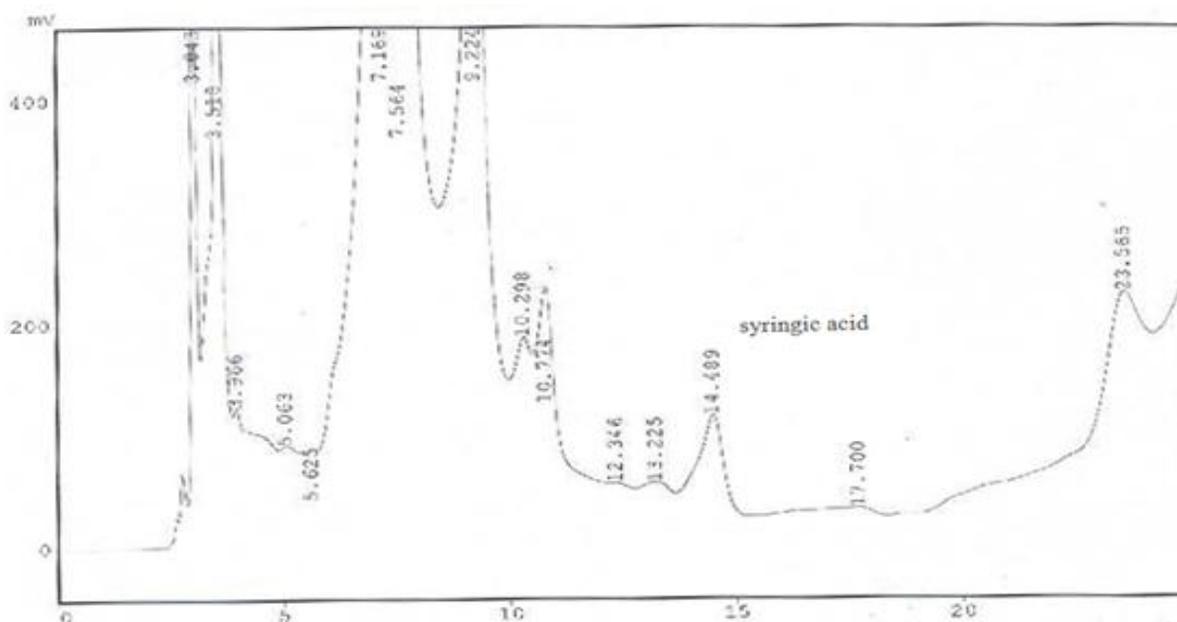


Figure 3 HPLC spectrum of the leaves crude extract of *Lawsonia inermis* at 280 nm absorbance using ethanol as solvent.

hydroxybenzoic acid (10.29 min), syringic acid (14.48 min), paracumic acid (23.56 min). Highest concentration of Gallic acid was reported from the leaves extract of *L. inermis* (3936.86 ng/g) this was followed by *A. indica* leaf (1548.42 ng/g), *F. carica* leaf (1172.79 ng/g), *L. sativum* seed (896.37 ng/g) and *N. sativa* seed (12.58 ng/g). Para-hydroxybenzoic acid comes after gallic acid detected in all seed and leaf extracts. The highest amount of syringic acid (1201.88 ng/g) was reported from the leaf extract of *L. inermis*, this was followed by the *Ficus carica* leaf (445.54 ng/g) and *N. sativa* seed (5.66 ng/g). The highest concentration of parahydroxy benzoic acid (797.8 ng/g) was recorded in extract of *O. europaea* plant. In all tested ten plants, parahydroxy benzoic acid was observed except in *B. sacra* plant extract (Figure 4). While the highest concentration (174.02 ng/g) of vanillic acid and syringic acid (21.76 ng/g) were depicted in *M. longifolia* plant extracts respectively. In *T. foenum-graecum*, gallic acid was observed with the highest concentration 244.87 ng/g at retention time 3.549 minute and syringic acid was the lowest 21.52 ng/g at retention time 13.464 minute while vanillic acid was not detected (Figure 5).

4 Discussion

The antimicrobial effect of the medicinal plants is well documented and reported variously. Among all tested plants, *N. sativa* extracts have shown significant antimicrobial effect against the tested pathogens. Similar results were reported by Mashhadian & Rakhshandeh (2005) when they tested the antibacterial and antifungal effects of *N. sativa* seeds extract against common and

hospital strains of *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and compared them with standard drugs such as clotrimazole, cloxacillin and gentamicin. Similarly, Niakan et al. (2006) studied antimicrobial potential of *N. sativa* seeds oil against two common *Staphylococcus aureus* strains and found significant inhibitory effect. This antimicrobial action could be attributed to the active ingredients especially thymoquinone and melanin (Zuridah et al., 2008). Since thymoquinone was found as the major component of this essential oil, it is claimed that it has great contribution in antimicrobial potential of the *N. sativa* seeds (Priya et al., 2004).

Further *L. inermis*, *B. sacra* and *T. foenum-graecum* plant extracts also showed great antimicrobial potential against pathogenic blood cancer isolates. The antimicrobial potential of three *Boswellia* species particularly against the Gram-positive bacteria was recorded very effective against *Corynebacterium sp.*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* pathogenic strains (Hasson et al., 2011). Essential oil of *M. longifoli* showed highest antimicrobial activity against *Salmonella*, *Klebsiella pneumonia* and *Staphylococcus aureus* strains supporting the observations of present study (Gulluce et al., 2007). Different solvents which included water, ethanol, methanol, ethyl acetate, n-hexane and diethyl ether were used for plant crude extraction of *O. europaea* and tested against pathogenic *Klebsiella pneumonia*, *Salmonella* and *Staphylococcus aureus* strains. The results were highly promising providing *O. europaea* as an effective medicinal plant (Hussain et al., 2014). In present study, all ten medicinal plants tested showed enormous

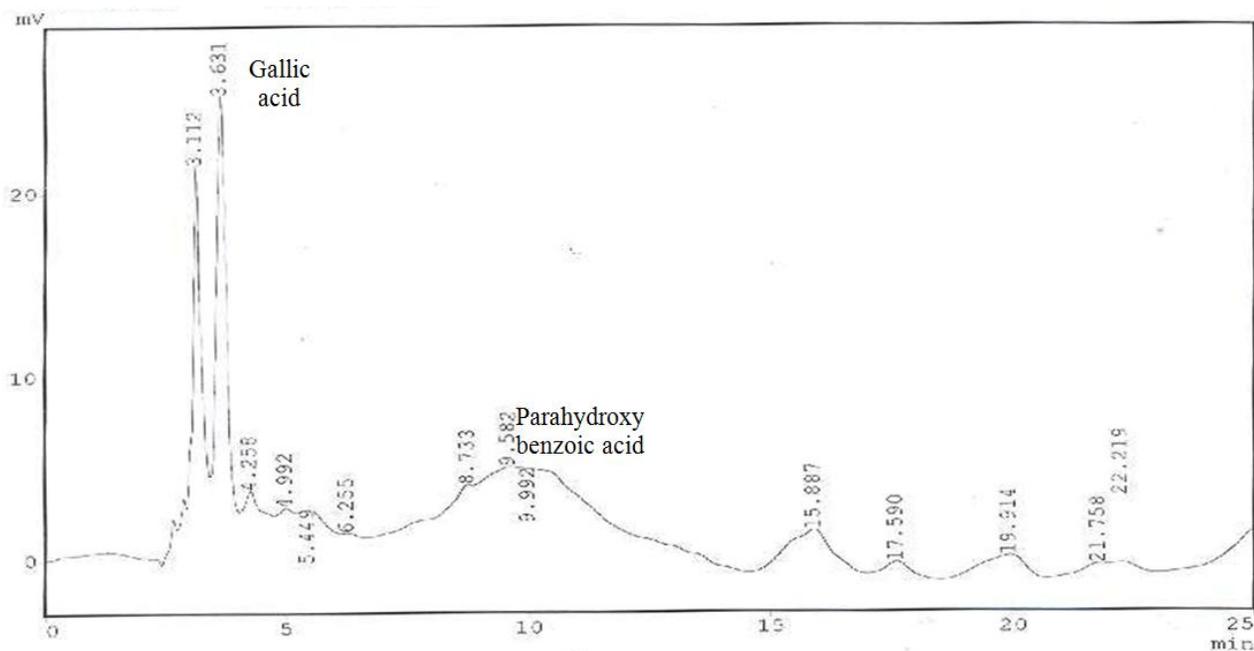


Figure 4 HPLC chromatogram of the studied *Boswellia sacra* recorded at 280 nm

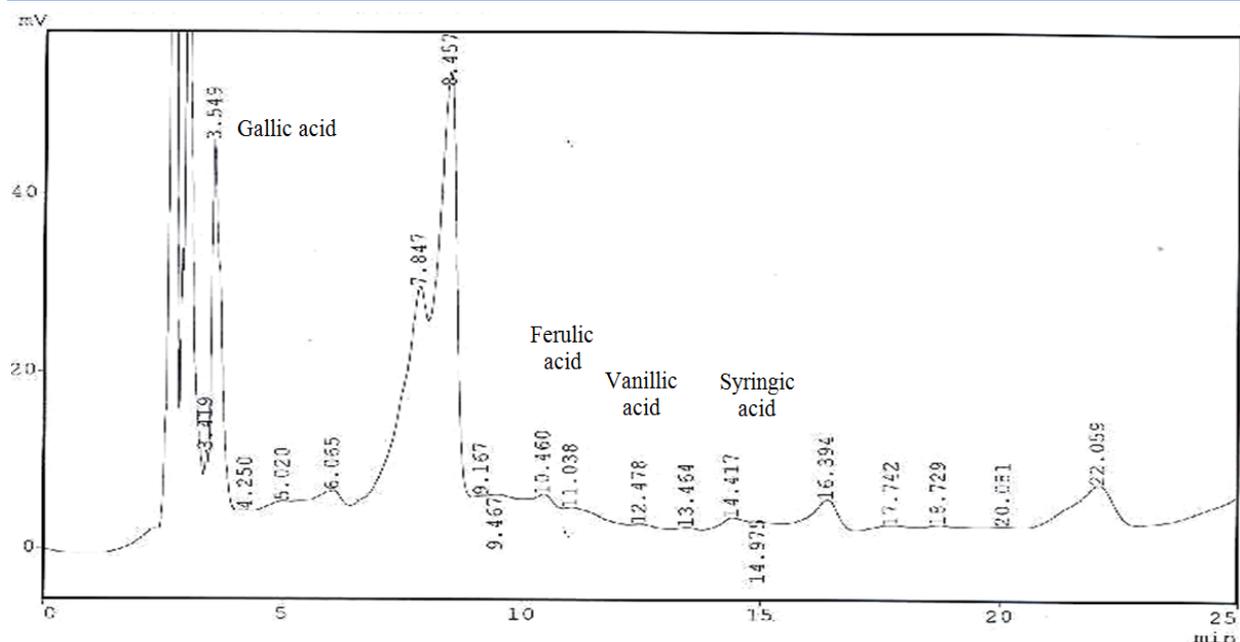


Figure 5: HPLC chromatogram of the studied *Trigonella foenum-graecum* seeds recorded at 280 nm

antimicrobial potential against diverse group of bacteria. Different range of antimicrobial potential of these crude extracts could be due to variation in environmental and genetic factors. In plants, unusual chemotypes and the nutritional values of different parts (seeds, leaves, roots or stem) could also influence the crude extracts and oil compositions leads to antimicrobial activity differences (Ozcan & Chalchat, 2002; Dubey et al., 2004; Kordali et al., 2005).

Medicinal plants have symbolized safety in contrast to the synthetic treatment (chemotherapy and radiotherapy). They play an essential role in the treatment of cancer. In cancer patients, the use of complementary and alternative medicine is the first desirable and recommended treatment with lesser side effects as compared to the synthetic chemical drugs (Ghavami et al., 2010).

This study also revealed a very interesting fact that all plant extracts were more effective on Gram positive than Gram negative bacteria. Gram negative bacteria have an effective natural permeability barrier in its outer membrane, which restricts the diffusion of amphipathic biochemical complexes (Tegos et al., 2002; Shan et al., 2007; Cueva et al., 2010).

The different biological activities depend on the plant secondary metabolites and regular consumption of these herbal products have serious consequences for health, it might be positive or negative (Mossini et al., 2009; Stobiecki & Kachlicki, 2011). Polyphenols and flavonoids are used for cure and

prevention of various diseases which are mainly associated with free radicals (Elansary et al., 2018). In present study, total phenolics content ranged from 8.651 ± 0.321 to 5232 ± 0.231 mg GAE/g (gallic acid equivalent) in the selected plant extracts. The total flavonoids content ranged between 4.023 ± 0.70 and 2282 ± 0.45 mg quercetin equivalent per g extracts. The entire plant kingdom contains phenolics and flavonoids which are important components of human and animal diet (Al-Jadidi & Hossain, 2015; Bianchi et al., 2018). The biological properties of the plants have been attributed to the presence of active compounds like phenolic compounds (gallic acid, proto-catechuic acid, vanillic acid, syringic acid, para-cumeric acid, para-hydroxybenzoic acid). The leaves of *L. inermis* contain 3936.86 ng/g of gallic acid followed by *A. indica* leaf (1548.42 ng/g), *Ficus carica* leaf (1172.79 ng/g). Gallic acid is the most prominent phytoconstituents present in this plant leaves that could be responsible for its antimicrobial activity (Aliu, 2007).

Phenolic acids have significant biological and pharmacological characteristics and proved highly benefit for human health against pathogenic infection. These biochemical compounds are vital components of the human food having antioxidant and anticancer property and play an important role to reduce oxidative stress in human being (Harris et al., 2007). In various previous studies, gallic acid and ferulic acid were identified and quantified in ethanol crude extracts successfully (Juliani & Simon, 2002; Laura et al., 2009). The phenolic compound gallic acid from plant crude extracts of *Mentha longifolia* and *Mentha spicata* found in

common but differed in the content of other detected individual phenolic compounds (Vlase et al., 2014). Ferulic acid was also found in the ethanolic extract of *Mentha longifolia* aerial parts, but could not be quantified due to lesser amount of concentration (Al-Mandeel, 2013; Benedec et al., 2013). In leaves of olive plants, different phenolics like tyrosol, vanillin, hydroxytyrosol, caffeic acid, vanillic acid & oleuropein were detected and reported variously. In olive fruit, vanillic acid, syringic acid, gallic acid and ferulic acid were also found (Kountouri et al., 2007; Sedef & Karakaya, 2009; Viola & Viola, 2009; Rababah et al., 2011). The phenolic acids were recorded quite susceptible to degradation when exposed to environmental stress like light, pH, temperature, humidity and oxygen (Bianco et al., 2001) that explains the difference in antimicrobial potential of these plants also supporting the results of present study. The highest antimicrobial potential of the methanol extracts observed in this study may be due to free hydroxyls that have the capability to combine and interact with the carbohydrates and proteins in the bacterial cell wall. They may be attached to enzyme sites rendering them inactive (Friedman & Jurgens, 2000; Moreno et al., 2006; Stalikas, 2007). Therefore, the total analysis of chemicals amounts detected from the same plant and their corresponding antioxidant and antimicrobial activities may vary widely, depending on external conditions applied. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process that plays an active role in metabolism of living cells (Kessler et al., 2003).

Conclusion

Studies suggested that herbal medicines have a great potential in combating leukaemia. These medicinal plants parts could be the best candidate for future leukaemia therapy with minimal adverse effects, easier availability, and better acceptability as compared to chemotherapy and probably they will provide more potent antileukaemic agents in future. The present paper is a contribution to the studies on the pharmaceutical and antimicrobial potential of different medicinal plants from Saudi region. The results of this study demonstrated that different natural antimicrobials of plant origin could effectively inhibit various pathogenic blood cancer isolates, thus providing a good alternative to the use of current antimicrobials. The study revealed that the highest inhibitory effect of *N. sativa* crude extract was found against *Streptococcus viridians*. Also, *Streptococcus viridian* and *Corynebacterium* strains were found more sensitive as compared to *Klebsiella pneumonia* that showed highest resistance in antimicrobial activity. Promising antimicrobial potential of *N. sativa*, *L. inermis*, *B. sacra* and *T. foenum-graecum* plants were notably observed. More importantly, these medicinal plants seem to be a good source of several important phytochemicals, such as phenolics and

flavonoids. Hence, further studies are needed to isolate pure compounds from the crude extract and to better understand the mechanism of such actions scientifically. These medicinal plant extracts also open the possibility of finding new clinically effective antibacterial compounds against multi-resistant pathogenic bacteria and pave the way toward the discovery of new antimicrobial drugs. Therefore, they could become a “preventive model” for infectious disease suppression and could be useful to improve the health of blood cancer cases.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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